

Influence of Persistent Inflammation in Follow-Up Biopsies After Antibody-Mediated Rejection in Kidney Transplantation

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SUPPLEMENTARY INFORMATION

Table S1. Demographic characteristics of patients with and without follow-up biopsy.

Table S2. Histological characteristics of the biopsy at ABMR diagnosis in patients with and without follow-up biopsy.

Table S3. Demographic characteristics of patients with and without persistent microvascular inflammation (MVI) in the follow-up biopsy.

Table S4. Histological characteristics of the biopsy at ABMR diagnosis in patients with and without persistent MVI.

Table S5. Death censored graft loss and MVI ≥ 1 in Follow-up Biopsy adjusted by histological characteristics.

Table S6. Death censored graft loss and histological variables adjusted by MVI ≥ 1 in Follow-up Biopsy.

Table S7. Histological characteristics and Death censored Graft loss, adjusted by time to rejection.

Table S1. Demographic characteristics of patients with and without follow-up biopsy.

	Patients with Follow-up Biopsy (n = 90)	Patients without Follow-up Biopsy (n = 26)	P value
Donor			
Age (years)	55.68 ± 13.61	51.19 ± 16.27	0.16
Gender (male)	41 (46)	18 (69)	0.03
DDKT	59 (66)	24 (92)	0.01
DCD	9 (15)	3 (13)	0.75
Recipient age (years)	49.58 ± 14.16	55 ± 12.71	0.08
Recipient gender (male)	55 (61)	14 (54)	0.51
Dialysis vintage (years)	2.5 [4.5]	4 [5]	0.66
Dialysis modality			0.98
Preemptive	15 (16)	4 (16)	
Hemodialysis	68 (76)	20 (76)	
Peritoneal dialysis	7 (8)	2 (8)	
ABMR < 1 year after KT (yes)	75 (83)	17 (65)	0.06
Hypertension (yes)	71 (79)	17 (65)	0.16
HCV (yes)	17 (19)	12 (46)	0.01
DM (yes)	15 (17)	5 (19)	0.77
Vasculopathy (yes)	16 (18)	3 (12)	0.45
ESKD etiology			0.41
ADPKD	12 (13)	5 (18)	
Urological	26 (29)	3 (12)	
Glomerulonephritis	19 (22)	7 (27)	
Diabetic nephropathy	9 (10)	3 (12)	
Nephroangiosclerosis	4 (4)	3 (12)	
Unknown	20 (22)	5 (19)	
Previous KT (any)	48 (53)	8 (31)	0.04
HLA mismatches	3.98 ± 1.62	4.50 ± 1.20	0.13
ABO incompatibility (yes)	12 (13)	4 (15)	0.79
Pre-transplant DSAs (any)	14 (16)	4 (15)	0.92

DSAs at ABMR diagnosis (any)	59 (70)	14 (54)	0.12
PRA > 50% (yes)	13 (14)	5 (19)	0.55
CF-CM at KT (positive)	15 (17)	2 (8)	0.13
Luminex at KT (positive)			
Class I	34 (39)	5 (19)	0.001
Class II	38 (42)	6 (23)	0.002
Induction Immunosuppression (yes)	88 (98)	24 (92)	0.18
Basiliximab	30 (33)	9 (35)	0.90
Rituximab	11 (12)	0 (0)	0.06
Thymoglobulin	54 (60)	14 (54)	0.65
Immunosuppression maintenance			
Tacrolimus	62 (69)	14 (54)	0.16
mTORi	33 (37)	8 (31)	0.58
Mycophenolate	72 (80)	20 (77)	0.73
Steroids	90 (100)	26 (100)	-
Need for dialysis at ABMR (yes)	26 (29)	6 (23)	0.56
ABMR treatment at diagnosis (yes)			
Plasma exchange	86 (96)	25 (96)	0.89
IVIg	18 (20)	4 (15)	0.60
Rituximab	80 (89)	21 (81)	0.28

Data are mean $\pm$ SD, median [IQR] or n (%), unless otherwise specified. ABMR, Antibody-mediated Rejection; KT, kidney transplantation; HCV, hepatitis C virus; DM, Diabetes Mellitus; ESKD, End-Stage Kidney Disease; ADPKD, Autosomal Dominant Polycystic Kidney Disease; HLA, Human Leucocyte Antigen; DSAs, Donor Specific Antibodies; PRA, Panel Reactive Antibodies; CF-CM, Flow Cytometry Crossmatch; mTORi, mTOR inhibitors; DDKT, Deceased Donor Kidney Transplantation; DCD, Donor after Cardiac Death; IVIg, Intravenous Immunoglobulin.

Table S2. Histological characteristics of the biopsy at ABMR diagnosis in patients with and without follow-up biopsy.

	Patients with Follow-up Biopsy (n = 90)	Patients without Follow-up Biopsy (n = 26)	P value
i	0.72 ± 0.92	0.54 ± 0.86	0.29
t	0.43 ± 0.78	0.5 ± 0.95	0.93
g	0.87 ± 0.97	0.77 ± 0.86	0.79
ptc	1.47 ± 0.96	1.3 ± 0.88	0.47
MVI (g + ptc)	2.33 ± 1.44	2.08 ± 1.02	0.48
C4d	72 (80%)	23 (88.5%)	0.32
ATN	29 (32.2%)	6 (23.1%)	0.37
Vascular inflammation	0.21 ± 0.57	0.15 ± 0.54	0.41
Total inflammation	0.61 ± 0.84	0.27 ± 0.53	0.06
TMA	5 (5.6%)	1 (3.8%)	0.73
ci	0.66 ± 0.79	0.65 ± 0.63	0.16
ct	0.64 ± 0.75	0.42 ± 0.64	0.68

Results are shown as mean ± SD for quantitative and qualitative variables. Qualitative variables, as C4d, are shown in absolute number and the percentage in brackets (% positive). i, interstitial inflammation; t, tubulitis; g, glomerulitis; ptc, peritubular capillaritis; ATN, acute tubular necrosis; atrophy; TMA, thrombotic microangiopathy; ci, interstitial fibrosis; ct, tubular atrophy.

Table S3. Demographic characteristics of patients with and without persistent microvascular inflammation (MVI) in the follow-up biopsy.

	MVI persistence (n = 64)	Without MVI (n = 26)	P value
Donor			
Age (years)	55.8 ± 13.7	55.4 ± 13.6	0.90
Gender (male)	27 (42)	14 (54)	0.36
DDKT	39 (61)	20 (77)	0.22
DCD	5 (13)	4 (20)	0.47
Recipient age (years)	48.8 ± 14.0	51.4 ± 14.7	0.44
Recipient gender (male)	38 (61)	16 (62)	0.96
Dialysis vintage (years)	4.5 [4.5]	3 [7]	0.43
Dialysis modality			0.43
Preemptive	12 (19)	3 (12)	
Hemodialysis	46 (72)	22 (85)	
Peritoneal dialysis	6 (9)	1 (4)	
ABMR < 1 year after KT (yes)	51 (80)	24 (92)	0.22
Hypertension (yes)	50 (78)	21 (81)	0.78
HCV (yes)	17 (11)	6 (23)	0.52
DM (yes)	8 (13)	7 (27)	0.10
Vasculopathy (yes)	10 (16)	6 (23)	0.40
ESKD etiology			0.65
ADPKD	10 (16)	2 (8)	
Urological	20 (31)	6 (23)	
Glomerulonephritis	13 (20)	6 (23)	
Diabetic nephropathy	5 (8)	4 (15)	
Nephroangiosclerosis	2 (3)	2 (8)	
Unknown	14 (22)	6 (23)	
Previous KT (any)	32 (50)	16 (62)	0.32
HLA mismatches	3.81 ± 1.74	4.39 ± 1.24	0.13
ABO incompatibility (yes)	10 (16)	2 (8)	0.32
Pre-transplant DSAs (any)	18 (28)	6 (23)	0.88
DSAs at ABMR diagnosis (any)	44 (76)	15 (58)	0.09
PRA > 50% (yes)	10 (16)	3 (12)	0.62
CF-CM at KT (positive)	12 (19)	3 (12)	0.34
Luminex at KT (positive)			
Class I	26 (42)	8 (31)	0.37
Class II	27 (42)	11 (42)	0.76
Induction Immunosuppression (yes)	63 (98)	25 (96)	0.51
Basiliximab	21 (33)	9 (35)	0.87
Rituximab	10 (16)	1 (4)	0.12
Thymoglobulin	39 (61)	15 (58)	0.78
Immunosuppression maintenance			
Tacrolimus	45 (70)	17 (65)	0.65
mTORi	24 (38)	9 (35)	0.80
Mycophenolate	51 (80)	21 (81)	0.91
Steroids	64 (100)	26 (100)	-
Need for dialysis at ABMR (yes)	18 (28)	8 (31)	0.80

ABMR treatment at diagnosis (yes)			
Plasma exchange	60 (94)	26 (100)	0.19
IVIg	13 (20)	5 (19)	0.91
Rituximab	58 (91)	22 (85)	0.41

Data are mean $\pm$ SD, median [IQR] or n (%), unless otherwise specified. ABMR, Antibody-mediated Rejection; KT, kidney transplantation; HCV, hepatitis C virus; DM, Diabetes Mellitus; ESKD, End-Stage Kidney Disease; ADPKD, Autosomal Dominant Polycystic Kidney Disease; HLA, Human Leucocyte Antigen; DSAs, Donor Specific Antibodies; PRA, Panel Reactive Antibodies; CF-CM, Flow Cytometry Crossmatch; mTORi, mTOR inhibitors; DDKT, Deceased Donor Kidney Transplantation; DCD, Donor after Cardiac Death; IVIg, Intravenous Immunoglobulin.

Table S4. Histological characteristics of the biopsy at ABMR diagnosis in patients with and without persistent MVI.

	MVI persistence (n = 64)	Without MVI (n = 26)	P value
i	0.77 $\pm$ 0.99	0.7 $\pm$ 0.9	0.822
t	0.42 $\pm$ 0.86	0.44 $\pm$ 0.75	0.71
g	0.65 $\pm$ 0.85	0.95 $\pm$ 1.01	0.22
ptc	1.34 $\pm$ 0.93	1.52 $\pm$ 0.98	0.44
MVI (g + ptc)	2 $\pm$ 1.33	2.47 $\pm$ 1.47	0.2
C4d	22 (84.6%)	50 (78.1%)	0.49
ATN	12 (46.2%)	17 (26.6%)	0.073
Vascular inflammation	0.38 $\pm$ 0.75	0.14 $\pm$ 0.47	0.037
Total inflammation	0.65 $\pm$ 0.8	0.59 $\pm$ 0.87	0.52
TMA	0	5 (7.8%)	0.14
ct	0.58 $\pm$ 0.76	0.67 $\pm$ 0.76	0.52
ci	0.64 $\pm$ 0.85	0.66 $\pm$ 0.78	0.89

Results are shown as mean $\pm$ SD for quantitative and qualitative variables. Qualitative variables, as C4d, are shown in absolute number and the percentage in brackets (% positive). i, interstitial inflammation; t, tubulitis; g, glomerulitis; ptc, peritubular capillaritis; ATN, acute tubular necrosis; atrophy; TMA, thrombotic microangiopathy; ci, interstitial fibrosis; ct, tubular atrophy.

Table S5. Death censored graft loss and MVI ≥ 1 in Follow-up Biopsy adjusted by histological characteristics.

Adjusted by	HR (95% CI)	P value
Crude	4.50 (1.35 – 15.0)	0.014
Acute cellular rejection (Yes)	3.51 (1.04 – 11.9)	0.044
Tubulitis ≥ 1 (Yes)	3.86 (1.15 – 12.9)	0.028
Tubulitis range	3.60 (1.08 – 12.0)	0.038
C4d + (Yes)	3.53 (1.04 – 12.0)	0.043
ATN (Yes)	5.71 (1.54 – 21.3)	0.009
Vascular inflammation ≥ 1 (Yes)	3.69 (1.09 – 12.5)	0.036
Total inflammation ≥ 1 (Yes)	3.60 (1.06 – 12.3)	0.04
Total inflammation range	3.76 (1.10 – 12.8)	0.034
TMA (Yes)	2.90 (0.84 – 10.0)	0.092
IFTA ≥ 1 (Yes)	3.56 (1.06 – 12.0)	0.04
IFTA range	3.43 (1.01 – 11.6)	0.047
Transplant Glomerulopathy ≥ 1 (Yes)	3.24 (0.94 – 11.2)	0.062

ATN, acute tubular necrosis; atrophy; TMA, thrombotic microangiopathy; IFTA: interstitial fibrosis and tubular atrophy.

Table S6. Death censored graft loss and histological variables adjusted by MVI ≥ 1 in Follow-up Biopsy.

	HR (95% CI)	P value
Acute cellular rejection (Yes)	1.32 (0.50 – 3.49)	0.581
Tubulitis ≥ 1 (Yes)	1.36 (0.59 – 3.12)	0.472
Tubulitis range	1.85 (1.04 – 3.29)	0.035
C4d + (Yes)	1.16 (0.39 – 3.50)	0.790
ATN (Yes)	9.19 (2.52 – 33.5)	0.001
Vascular inflammation ≥ 1 (Yes)	1.32 (0.38 – 4.53)	0.661
Total inflammation ≥ 1 (Yes)	1.25 (0.79 – 1.98)	0.338
Total inflammation range	1.76 (0.80 – 3.86)	0.158
TMA (Yes)	0.65 (0.09 – 4.85)	0.675
IFTA ≥ 1 (Yes)	1.56 (0.74 – 3.30)	0.247
IFTA range	1.46 (1.02 – 2.09)	0.040
Transplant Glomerulopathy ≥ 1 (Yes)	2.66 (1.13 – 6.24)	0.025

ATN, acute tubular necrosis; atrophy; TMA, thrombotic microangiopathy; IFTA: interstitial fibrosis and tubular atrophy.

Table S7. Histological characteristics and Death censored Graft loss, adjusted by time to rejection.

	Crude		Adjusted by Time to rejection	
(A) Biopsy at ABMR diagnosis	OR (95% CI)	P value	OR (95% CI)	P value
Acute cellular rejection (Yes)	2.21 (1.07 – 4.58)	0.033	2.15 (1.03 – 4.50)	0.042
Tubulitis (Yes)	1.85 (1.30 – 2.65)	0.001	1.52 (1.03 – 2.23)	0.033
Tubulitis range	2.10 (1.04 – 4.26)	0.039	1.44 (0.68 – 3.05)	0.338
Glomerulitis	0.92 (0.64 – 1.34)	0.678	0.94 (0.64 – 1.38)	0.747
Peritubular Capillaritis (Yes)	1.56 (1.09 – 2.24)	0.016	2.99 (1.26 – 7.09)	0.013
MVI (g + ptc) ≥ 1 (Yes)	1.19 (0.93 – 1.51)	0.161	1.15 (0.89 – 1.49)	0.279
C4d + (Yes)	1.23 (0.42 – 3.57)	0.709	1.36 (0.46 – 3.97)	0.576
ATN (Yes)	0.80 (0.38 – 1.67)	0.550	1.06 (0.49 – 2.28)	0.879
vascular inflammation ≥ 1 (Yes)	0.76 (0.36 – 1.63)	0.486	0.79 (0.36 – 1.70)	0.544
Total inflammation ≥ 1 (Yes)	1.07 (0.66 – 1.74)	0.782	0.90 (0.56 – 1.44)	0.659
TMA (Yes)	0.82 (0.11 – 6.02)	0.846	0.93 (0.13 – 6.86)	0.946
IFTA ≥ 1 (Yes)	1.62 (1.09 – 2.40)	0.017	1.35 (0.89 – 2.06)	0.161
IFTA range	2.30 (1.16 – 4.56)	0.017	1.87 (0.92 – 3.77)	0.083

	Crude		Adjusted by Time to rejection	
(B) Follow-up Biopsy	OR (95% CI)	P value	OR (95% CI)	P value
Acute cellular rejection (Yes)	1.54 (0.58 – 4.03)	0.384	1.46 (0.56 – 3.84)	0.441
Tubulitis ≥ 1 (Yes)	1.92 (1.09 – 3.36)	0.023	1.68 (0.95 – 2.97)	0.074
Tubulitis range	2.88 (1.24 – 6.69)	0.014	2.57 (1.09 – 6.02)	0.030
Glomerulitis ≥ 1 (Yes)	1.06 (0.72 – 1.54)	0.774	1.10 (0.74 – 1.66)	0.630
Peritubular Capillaritis ≥ 1 (Yes)	2.99 (1.27 – 7.07)	0.012	2.99 (1.26 – 7.09)	0.013
MVI (g + ptc) ≥ 1 (Yes)	4.50 (1.35 – 15.0)	0.014	3.61 (1.07 – 12.2)	0.039
C4d + (Yes)	1.77 (0.60 – 5.19)	0.297	1.87 (0.63 – 5.53)	0.257
ATN (Yes)	4.56 (1.53 – 13.6)	0.006	4.30 (1.39 – 13.3)	0.011
vascular inflammation ≥ 1 (Yes)	0.11 (0.00 – 663.7)	0.665	0	0.999
Total inflammation ≥ 1 (Yes)	1.58 (1.02 – 2.46)	0.040	1.27 (0.80 – 2.01)	0.312
TMA (Yes)	3.70 (1.50 – 9.15)	0.005	4.60 (1.81 – 11.7)	0.001
IFTA ≥ 1 (Yes)	1.62 (1.14 – 2.32)	0.008	1.45 (1.01 – 2.08)	0.041
IFTA range	2.30 (0.87 – 6.09)	0.093	2.36 (0.89 – 6.28)	0.085
Transplant Glomerulopathy ≥ 1 (Yes)	2.24 (1.33 – 3.77)	0.003	1.92 (1.10 – 3.34)	0.022
Transplant Glomerulopathy range	4.39 (1.96 – 9.83)	0.000	3.26 (1.38 – 7.66)	0.007

MVI, microvascular inflammation; ATN, acute tubular necrosis; atrophy; TMA, thrombotic microangiopathy; IFTA: interstitial fibrosis and tubular atrophy.